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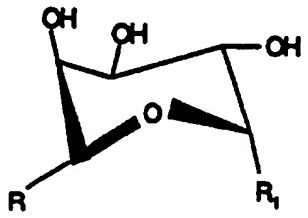
(54) Title: FUCOPEPTIDES

(57) Abstract

Compounds of formula (I) wherein R is CH_3 and R_1 is a peptidic residue or R_1 is OH and R is a peptidic residue have pharmacological activity as sialyl Lewis X mimetics, e.g. in the prevention or treatment of disorders or diseases which are mediated by the binding of selectins in cellular adhesion.



(I)

Fucopeptides

The present invention relates to fucopeptides, a process for their production, their use as a pharmaceutical and pharmaceutical preparations containing them.

It is widely accepted that a family of receptors, the selectins, are involved in the recognition of various circulating cells by the endothelium and platelets and play a role in certain diseases including cancer, autoimmune disorders, inflammation, atherosclerosis and blood clotting. There are three known members of this family: L-selectin, P-selectin and E-selectin.

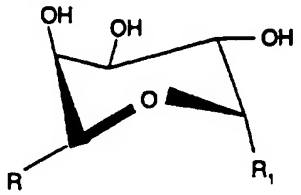
E-selectin (also designated endothelial leukocyte adhesion molecule, ELAM-1) is a cell surface protein inducibly expressed in endothelial cells. For example, its production is increased on vascular endothelial cells when adjacent tissue has been damaged or invaded by a microorganism. E-selectin recognizes sialyl Lewis X (SLe^x) which is a cell surface carbohydrate ligand found on neutrophils and monocytes, anchored onto the outer membrane thereof by integral membrane glycoproteins and/or glycolipids. SLe^x mediates binding of neutrophils and monocytes to the activated vascular endothelial cells by binding to E-selectin, so that these leukocytes may diffuse into the damaged tissue.

However, there are many situations in which the recruitment of leukocytes by adhesion to the endothelial cells is abnormal and in excess, and the end result is tissue damage instead of repair.

Although SLe^x has been considered to be potentially useful as an anti-inflammatory agent and its synthesis on large scales has been developed for clinical evaluation, this natural saccharide can only be used as an injectable form in cases presenting with acute symptoms as it is orally inactive and has a short half-life in blood.

Thus, there is a need for compounds which can interfere with binding of the ligands to the selectins and prevent the initial cellular adhesion process.

According to the invention, there is provided a compound of formula I



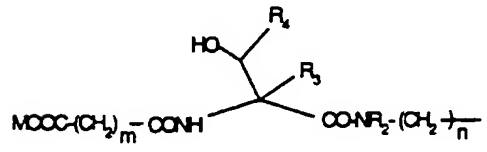
I

wherein

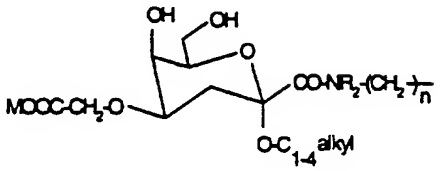
i) R is CH_3, and

either

R_1 is a radical of formulae (a_1) or (a_2)



(a_1)



(a_2)

wherein

m is 2 or 3;

n is 2 or 3;

M is a cation;

R_2 is H or a saturated or unsaturated hydrocarbon residue with up to 20 carbon atoms, optionally bearing in ω position a formyl or a C_{1-4} alcohol acetal or C_{2-4} diol acetal group;

R_3 is H, $-CH_2OH$ or $-CH_2CH_2OH$; and

R_4 is H, C_{1-4}alkyl, $-CH_2OH$, $-CH_2CH_2OH$ or $-CH_2CH_2CH_2OH$ with the provisos that

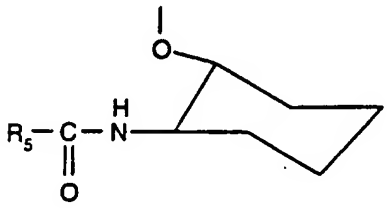
1) one of R_3 and R_4 is H, and

2) when R_4 is H, R_3 is $-CH_2OH$ or $-CH_2CH_2OH$, and

3) when R_3 is H, R_4 is CH_3, $-CH_2OH$, $-CH_2CH_2OH$ or $-CH_2CH_2CH_2OH$;

or

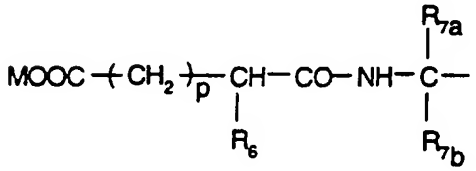
R_1 is a radical of formula (b)



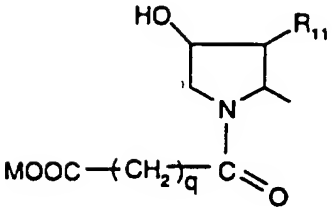
(b)

wherein

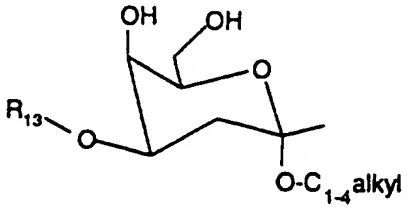
R_5 is



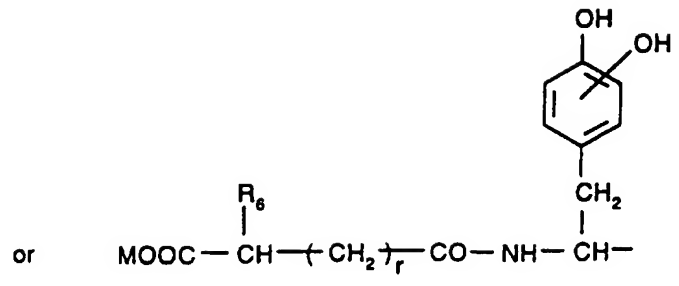
(b_1)



(b_2)



(b_3)



or (b4)

wherein

p is 1 or 2;

q is 2 or 3;

r is 1 or 2;

R_6 is H, NH_2 or $-NHR_x$ wherein R_x is an amino protecting group;

R_{7a} is $-CH_2OH$, $-CH_2CH_2OH$ or $-CH(OH)-CH_2OH$ and R_{7b} is H or each of R_{7a} and R_{7b} is CH_2OH;

R_{11} is H or -OH;

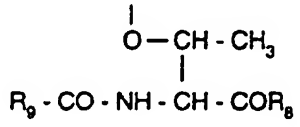
R_{13} is $-(CH_2)_j-COOM$ or $-SO_3M$ wherein j is 1, 2 or 3; and

M is as defined above;

the second hydroxy substituent of the phenyl group in (b_4) being in either meta position;

or

R_1 is a radical of formula (c)

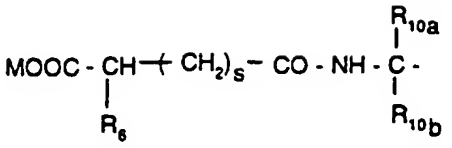


(c)

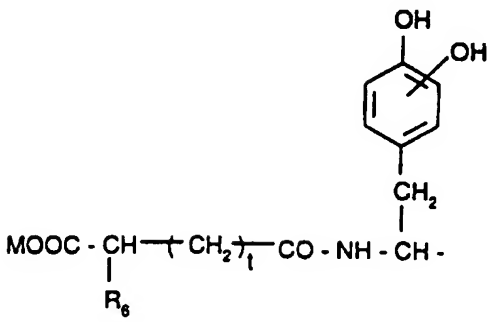
wherein

R_8 is OM_1, OR_{14}, R_s-R_p or $-NHR_y$ wherein M_1 is a cation, R_{14} is a saturated or unsaturated hydrocarbon residue, R_s is a spacer group, R_p is a phosphatidyl residue and R_y is a saturated or unsaturated lipophilic residue; and

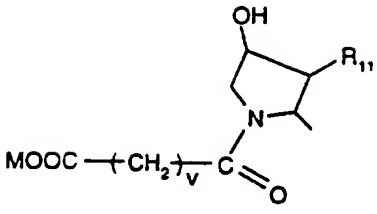
R_9 is



(c_1)

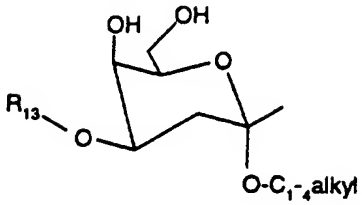


(c_2)



(c_3)

or



(c_4)

wherein

s is 1 or 2;

t is 1 or 2;

v is 2 or 3;

M, R_6, R_{11} and R_{13} are as defined above; and

R_{10a} is $-CH_2OH$, $-CH_2CH_2OH$ or $-CH(OH)-CH_2OH$ and R_{10b} is H

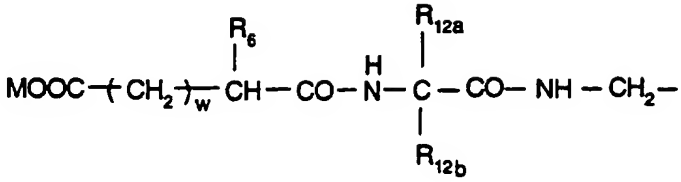
or each of R_{10a} and R_{10b} is CH_2OH;

the second hydroxy substituent of the phenyl group in (c_2) being in either meta position;

or wherein

ii) R_1 is OH, and

R is a radical of formula (d)



(d)

wherein

w is 1 or 2;

R_{12a} is -CH(OH)-$(CH_2)_x$-OH and R_{12b} is H or each of R_{12a} and R_{12b} independently is -CH_2OH or -CH_2CH_2OH;

x is 2 or 3; and

R_6 and M are as defined above.

M may be H^+ or any salt forming cation for a carboxy or sulfate which maintains the water-solubility of the compound of formula I, e.g. a monovalent or one equivalent of a polyvalent cation, for example an alkali metal ion such as lithium, sodium or potassium, an alkaline earth cation such as calcium or magnesium, as well as zinc, iron and aluminium ions and the ammonium (NH^+_4) ion. M is preferably H^+, Li^+ or Na^+. It is preferred that the cation M be a pharmaceutically acceptable cation. When the cation is polyvalent, an appropriate number of molecules of formula I or a mixture of compounds of formula I and one or more appropriate anions such as acetate, chloride, carbonate and the like are also present. M_1 may independently have one of the significance given above for M, preferably identical to M.

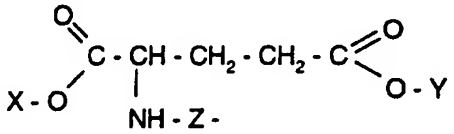
When R_x is an amino protecting group, it may be such a group as disclosed in "Protective Groups in Organic Synthesis" T.W. Greene, J. Wiley & Sons NY, 2nd ed., chapter 7, 1991, and references therein, preferably a pharmaceutically acceptable amino protecting group, particularly tert.-butoxy-carbonyl or benzyloxycarbonyl.

When R_2 in the radical of formula (a_1) is a saturated or unsaturated hydrocarbon residue, it may be e.g. C_{1-20}alkyl, C_{2-20}alkenyl or C_{2-20}alkynyl. Examples of R_2 bearing a formyl group include e.g. 2-oxo-ethyl, 3-oxo-propyl, 5-oxo-pent-3-enyl, 8-oxo-octyl and 10-oxo-dec-4-enyl. When R_2 is an alkyl or alkenyl acetal group formed from a C_{1-4}alcohol or C_{2-4}diol, it may be e.g. any of the acetals preparable from the above aldehydes using C_{1-4}alcohols or C_{2-4}diols, e.g. methanol, ethanol, isopropanol, sec.-butanol, n-butanol, ethylene glycol, propylene glycol, 2,3-butanediol,1,4-butanediol or 1,3-butanediol. R_2 is preferably H.

Any C_{1-4}alkyl as R_4 in the radical of formula (a_1) is preferably CH_3.

When R_8 is R_s-R_p or NHR_y, the resulting compound of formula I may be or is suitably utilized in a liposomal preparation as part of the liposome membrane as a means for administering said compound. The spacer group R_s is a residue which links the carbonyl to the oxygen of the phosphatidyl residue, e.g. a hydrocarbon residue.
The phosphatidyl residue is a glycerophosphate esterified with saturated and/or unsaturated fatty acids, e.g. myristic, palmitic, stearic, palmitoleic or oleic acid.

R_y may be a saturated or unsaturated aliphatic residue optionally comprising or interrupted by a functional group e.g. -CO-, e.g. a residue based on a dicarboxylic acid diester bearing saturated or unsaturated fatty aliphatic residues. R_y is preferably



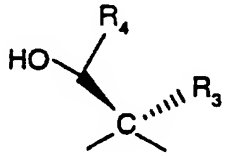
wherein each of X and Y independently is C_{8-20}alkyl or C_{8-20}alkenyl and Z is a bridging group, e.g. a polyethyleneoxy group, preferably a poly-(3-50, preferably 3-15)-ethyleneoxy group, or a -C_{1-4}alkylene-CO- or -phenylene-CO-group.

In the compounds of formula I, the following significances are preferred either individually or in any combination or sub-combination:

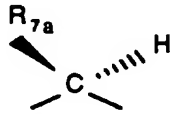
1. R is CH_3 and R_1 is a radical of formula (a_1).
2. In the radical of formula (a_1) R_2 is H.
3. In the radical of formula (a_1) R_3 is H and R_4 is -CH_2OH.
4. In the radical of formula (a_1) R_3 is H and R_4 is CH_3.
5. R is CH_3 and R_1 is a radical of formula (b).
6. In the radical of formula (b), R_5 is a radical (b_1).
7. In the radical (b_1) R_{7a} is CH_2OH or CH(OH)-CH_2OH and R_{7b} is H.
8. In the radicals (a_2) and (b_3), the group -OC_{1-4}alkyl is preferably OCH_3.
9. R is CH_3 and R_1 is a radical of formula (c).
10. In the radical of formula (c) R_8 is C_{1-6}alkoxy, preferably methyl or ethyl.
11. In the radical of formula (c), R_9 is a radical (c_1).
12. In the radical (c_1), R_{10a} is CH(OH)-CH_2OH and R_{10b} is H.
13. In the radical of formula (c), R_9 is a radical (c_2).
14. In the radical (c_2), R_6 is NH_2.
15. R_1 is OH and R is a radical of formula (d).
16. In the radical of formula (d), R_{12a} is CH(OH)-$(CH_2)_x$-OH and R_{12b} is H.

The compounds of formula I may comprise one or more asymetric carbon atoms. It will be understood that the present invention includes all individual isomeric forms, enantiomers and diastereoisomers as well as mixtures, e.g. racemates, unless otherwise stated.

In the radical of formula (a_1), the asymetric carbon atom bearing R_3 has preferably following configuration:

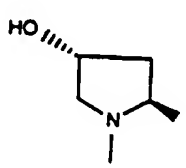


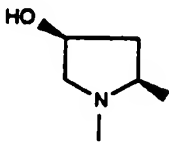
In the radical of formula (b_1), when the carbon atom bearing R_{7a} and R_{7b} is asymetric (i.e. R_{7b} is H), it preferably has following configuration:

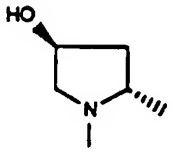


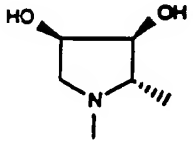
The same applies to the asymetric carbon atom bearing R_{10a} when R_{10b} is H in residue (c_1).

In the radicals (b_2) and (c_3), the pyrrolidinyl moiety preferably has following configuration:









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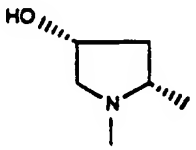
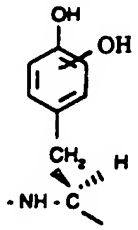
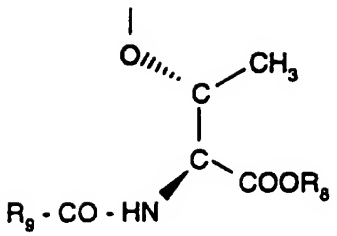


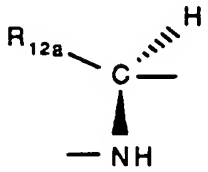
In the radicals (b_4) and (c_2) the asymetric carbon atom bearing the substituted benzyl moiety preferably has following configuration:



The radical of formula (c) has preferably following stereochemistry:



In the radical of formula (d), when R_{12b} is H, the asymetric carbon atom bearing R_{12a} has preferably following configuration:



The present invention also includes a process for the production of the compounds of formula I. They may be produced by analogy to known methods. The compounds of formula I may be produced for example by removing at least one protecting group which is present in a compound of formula I in protected form, e.g. amino and/or hydroxy protected form.

The compounds of formula I in protected form are mainly compounds wherein the hydroxy group(s) present in the fucose moiety is (are) protected. Groups which can be employed in the present invention to block or protect the hydroxy group are well-known to those skilled in the art and, preferably, said groups can be removed, if desired, by methods which do not result in any appreciable destruction of the remaining portion of the molecule, for example, by chemical or enzymatic hydrolysis, treatment with chemical reducing agents under mild conditions, irradiation with ultraviolet light or catalytic hydrogenation. Hydroxy-protecting (blocking) groups which are advantageously used are those which are common in carbohydrate chemistry especially for primary alcohols, secondary alcohols and vicinal cis and trans diols.

Suitable hydroxy-protecting groups may be, for example, acyl groups such as acetyl, trichloroacetyl, phenoxycarbonyl, benzyloxycarbonyl, benzhydryloxycarbonyl, trityl-

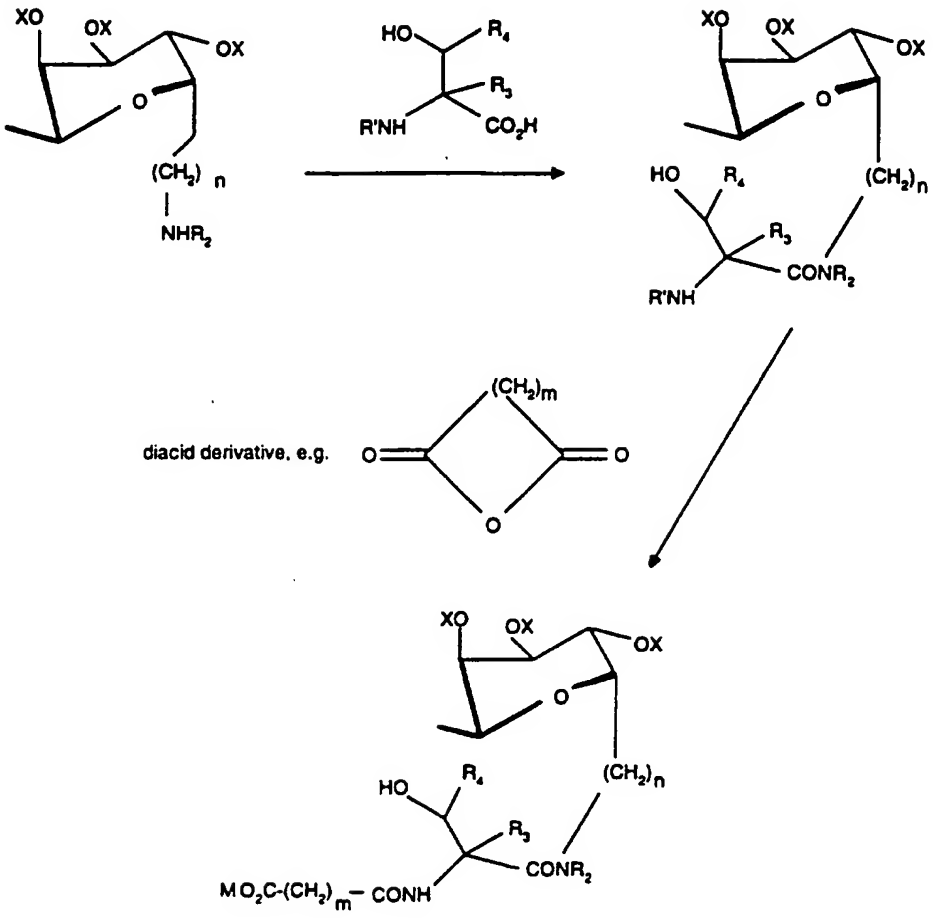
oxycarbonyl and 2,2,2-trichloroethoxycarbonyl, ether groups such as methoxymethyl, benzyloxymethyl, allyl, benzyl, p-methoxybenzyl, p-nitrobenzyl, benzhydryl, trityl or triorganosilyl groups such as tri(C_1-C_6) alkylsilyl (e.g. trimethylsilyl, triethylsilyl), tri-isopropylsilyl, isopropyldimethylsilyl, t-butydimethylsilyl, methyldiisopropylsilyl or methyldi-t-butylsilyl), t-butyl-diphenylsilyl, triarylsilyl (e.g. triphenylsilyl, tri-p-xylylsilyl) or triaralkylsilyl (e.g. tribenzylsilyl). Examples of these and other suitable hydroxy-protecting groups e.g. for the protection of 1,2- or 1,3-dihydroxy groups, for example cyclic ether groups such as optionally substituted methylene acetal or ethylidene acetal and methods for their formation and removal are known in the art, e.g., see Protective Groups in Organic Synthesis, second ed., T.W. Greene and P.G.M. Wuts, John Wiley & Sons, New York, 1991, Chapter 2 and references therein.

Compounds of formula I wherein R_6 is NHR_x may also be converted in compounds of formula I wherein R_6 is NH_2 by removal of the amino protecting group R_x.

The compounds of formula (I) thus obtained may be recovered in free form or in salt form.

Compounds of formula I in protected form wherein R is CH_3 and R_1 is a radical of formula (a), used as starting materials may be produced, e.g. as indicated in Scheme 1.

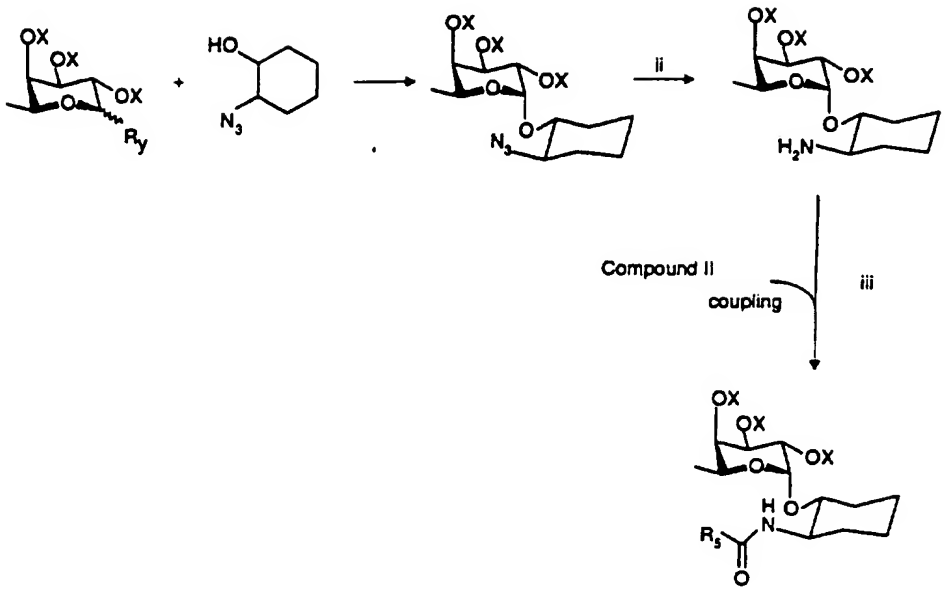
Scheme 1



X is a hydroxy protecting group, e.g. as indicated above. R' is a leaving group, e.g. an amino protecting group, for example Boc or Fmoc. Preferably the starting materials are used as specific enantiomers in order to obtain the compounds of formula I with the desired configuration.

Compounds of formula I in protected form wherein R is CH_3 and R_1 is a radical of formula (b) used as starting materials may be prepared, e.g. as indicated in Scheme 2.

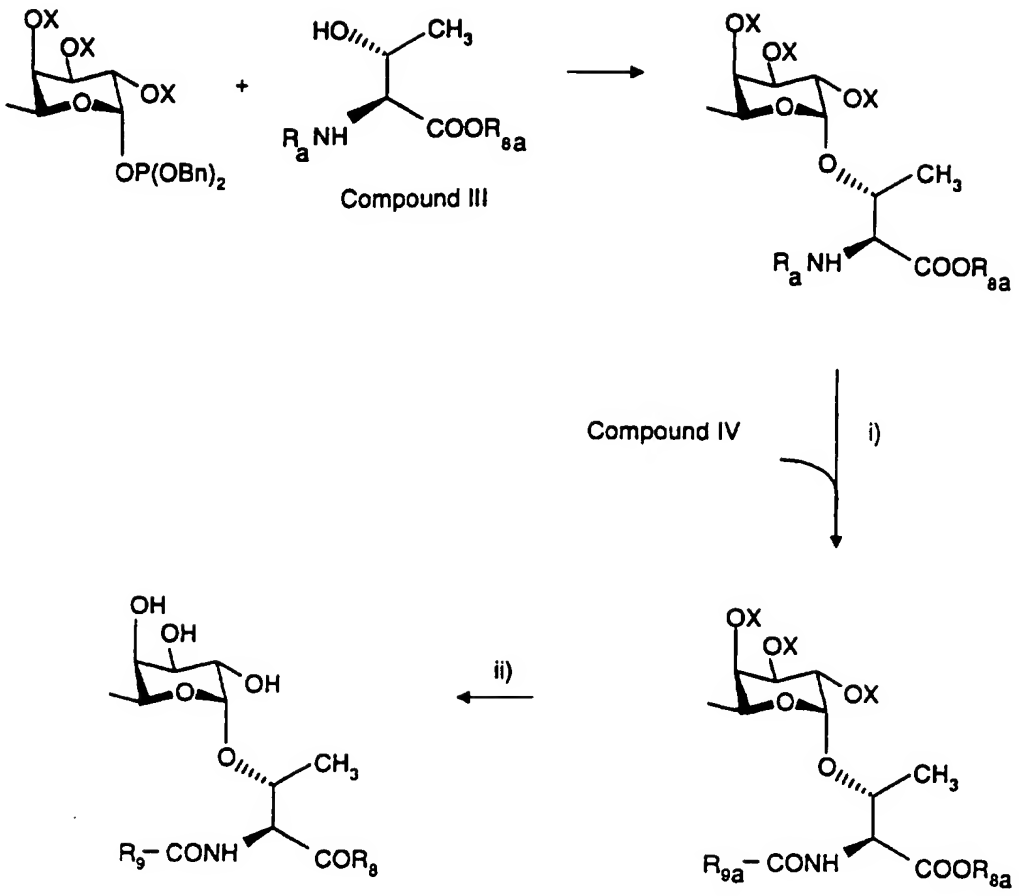
Scheme 2



R_y is a leaving group, e.g. halogen, preferably F. Step ii is a reduction which is intended to include well-known reduction procedures for the azido group such as reaction with a phosphine, e.g. triphenylphosphine, or a hydride, e.g. lithium aluminium hydride. Step iii is an amide bond coupling e.g. as known in the art of peptide chemistry. Compound II may be selected e.g. from Asp-Ser-OH, Glu-Ser-OH, Glu-(α-hydroxymethyl)Ser-OH in protected form, e.g. Compound 10. Step iii may also comprise coupling with an appropriate amino acid in protected form followed by reacting with a diacid derivative as disclosed in Scheme 1.

Compounds of formula I in protected form wherein R is CH_3 and R_1 is a radical of formula (c) may be prepared e.g. as indicated in Scheme 3.

Scheme 3

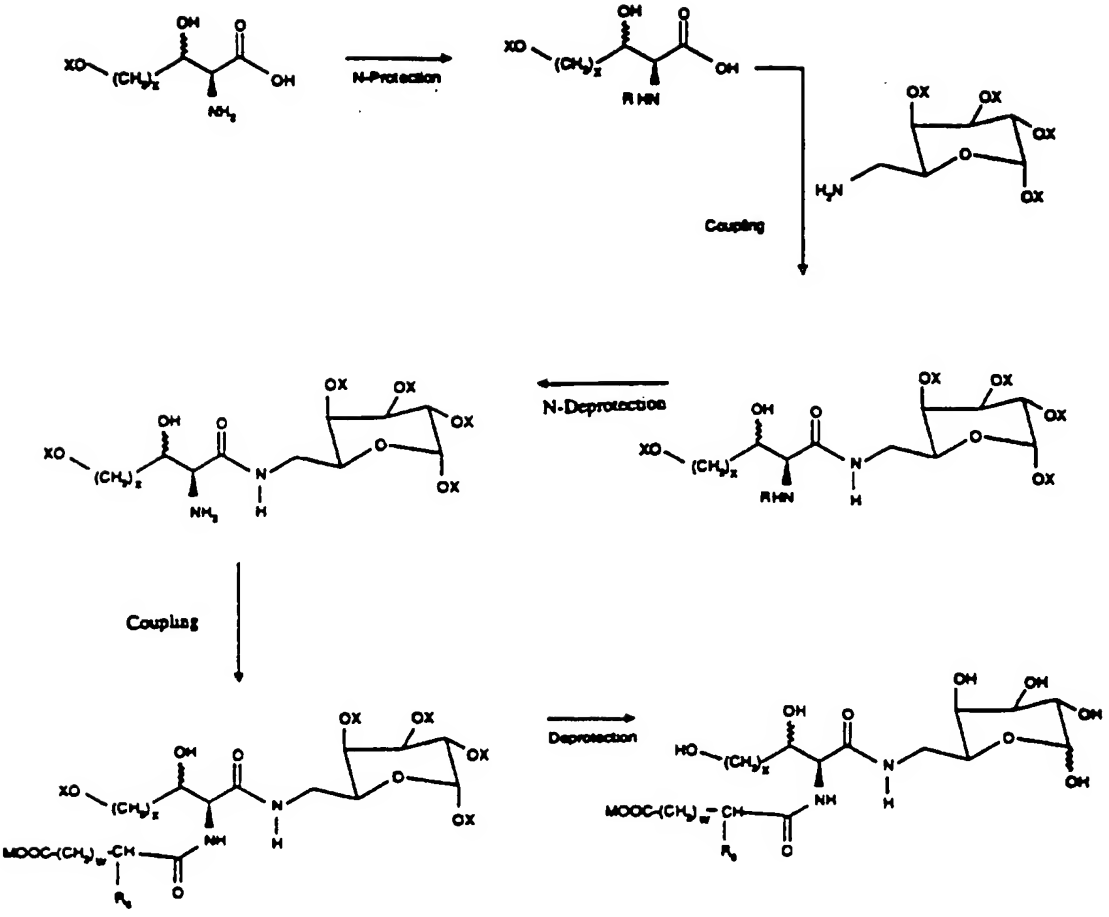


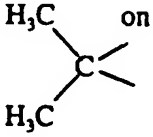
R_{8a} is C_{1-6}alkyl. R_a is an amino protecting group. Compound IV may be selected e.g. from 2-amino-3,4-dihydroxy-butyric acid, α-hydroxymethyl serine or Glu-Tyr-OH in protected form, e.g. (2S,3R)-N-Boc-2-amino-4-benzyloxy-3-hydroxybutyric acid. $R_{9a}CO$ is the amino acid residue (optionally completed with a diacid residue) of Compound IV. Steps i) and ii) are amide bond coupling reactions effected according to standard procedures. Step ii) may also comprise coupling with an appropriate amino acid in protected form followed by reacting with a diacid derivative, e.g. as disclosed in Scheme 1. Step ii) may further also comprise the conversion of $-COOR_{8a}$ into $-COR_8$, e.g. into an acid, acid salt, lipophilic ester or lipophilic amide, e.g. as disclosed in Examples 44 and 45. Compound III may be used in the form of one or another of the individual enantiomers or in the form of mixtures. Above Scheme 3 illustrate the

preparation of a compound of formula I wherein R is CH_3 and R_1 is a radical of formula (c) with the preferred configuration.

Compounds of formula I in protected form wherein R_1 is OH and R is a radical of formula (d) wherein R_{12a} is $CH(OH)-(CH_2)_x-OH$, used as starting materials, may be produced e.g. as indicated in Scheme 4.

Scheme 4



Preferably 2 vicinal X groups (hydroxy protecting groups) form together  on the fucose moiety.

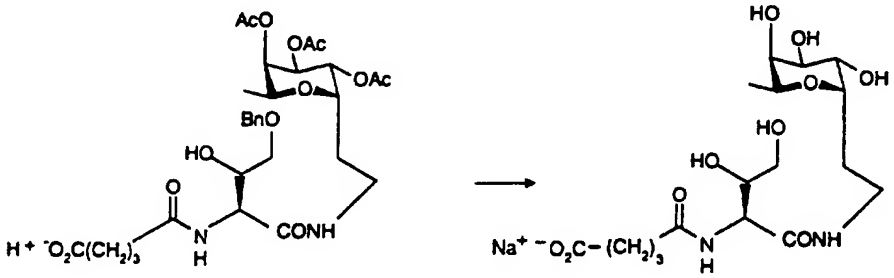
The above reactions may be effected in analogy with known methods, e.g. as described in the following examples. Insofar as the production of the starting materials is not particularly described, the compounds are known or may be prepared analogously to methods known and practiced in the art.

The following examples are illustrative of the invention. All temperatures are in °C.

Following abbreviations are used:

Ac	=	$-COCH_3$
Bn	=	benzyl
Boc	=	t.-butoxycarbonyl
Fmoc	=	9-fluorenylmethoxycarbonyl
DAST	=	diethylaminosulphur trifluoride
EDCI	=	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
EDAC	=	1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide
DMF	=	dimethylformamide
HOBT	=	1-hydroxybenzotriazole
TFA	=	trifluoroacetic acid

Example 1:

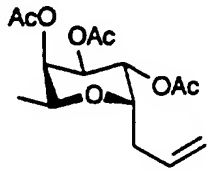


Compound 6 is deprotected by treatment with hydrogen gas over a palladium/carbon catalyst, followed by treatment with sodium methoxide to give the sodium salt of Compound 7.

^{1}H NMR (500 MHz, D_2O) δ 1.16 (d, J = 6.3 Hz, 3 H), 1.75-1.92 (m, 4H), 2.35 (m, 1 H), 3.20 (m, 1 H), 3.43 (m, 1H), 3.55 (dd, J = 6.0, 12.0 Hz, 1 H), 3.67 (dd, J = 3.0, 12.0 Hz, 1 H), 3.73 (m, 2 H), 2.84-4.02 (m, 4 H); ^{13}C NMR (125 MHz, D_2O) δ 16.6, 21.6, 24.5, 34.3, 35.4, 37.5, 56.5, 63.4, 68.2, 68.7, 70.8, 71.9, 72.6, 74.4, 166.8, 172.5, 177.0; electrospray mass m/z 423 [$(MH)^+$; calculated for $C_{17}H_{31}O_{10}N_2$: 423].

Compound 6, used as starting material, may be prepared as follows:

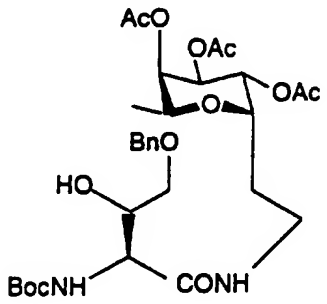
a) Fucose tetraacetate is treated with allyl trimethyl silane and boron trifluoride etherate in dry acetonitrile at room temperature to give Compound 2 (the ratio α:β is greater than 10:1). Kozikowsky, A.P. and Sorgi, K.L. Tetrahedron Lett. (1983), 24: 1563.



2

b) Compound 2 is ozonolyzed by reaction with ozone in the presence of triphenyl phosphine. The product aldehyde is then subjected in situ to reductive amination by treatment with hydrogen gas over a palladium/carbon catalyst in the presence of ammonium acetate to give the corresponding fucose tetraacetate bearing a 2-amino-ethyl group (Compound 3).

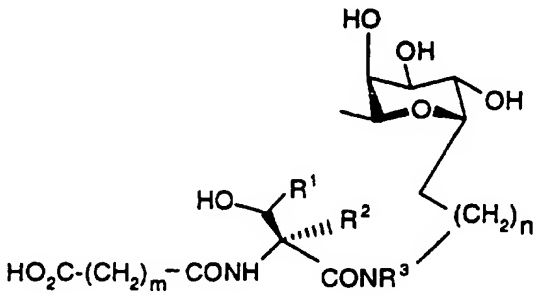
c) Compound 3 as obtained above is coupled with (1S,2R)-2-N-Boc - amino-4-benzyloxy-3-hydroxy butyric acid (prepared from glycine and O-benzylglyco-aldehyde by a threonine aldolase-catalyzed reaction according to Wong et al. Tetrahedron Lett. 1995, 36, 4081) in 1-(3-dimethylaminopropyl)-3-ethylcarbodiionide hydrochloride solution to form the amide Compound 5



5

d) The protecting group is removed from Compound 5 by treatment with ethyl acetate in acidic solution (4N HCl). The deprotected product is then treated in situ with glutaric anhydride (in triethylamine buffer) to obtain Compound 6.

By following a procedure in analogy with that of Example 1 above, the compounds of formula

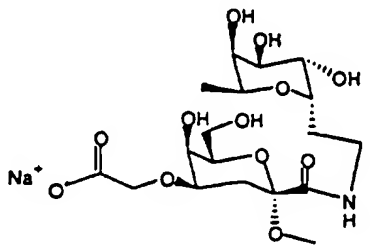


wherein R_1, R_2, n and m have the significances as indicated in Table 1 below, may be prepared.

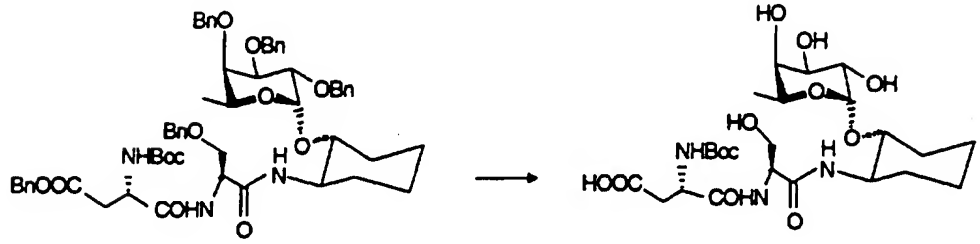
Table 1

Ex.	R_1	R_2	n	m
2	CH_2OH	H	2	3
3	CH_3	H	1	3
4	H	CH_2OH	1	3
5a	CH_3	H	1	2
5b	CH_2CH_2OH	H	1	2
5c	CH_2CH_2OH	H	1	3

By following a procedure in analogy with that of Example 1 but using the appropriate starting materials, the compound of Example 6 may be obtained:



Example 7:



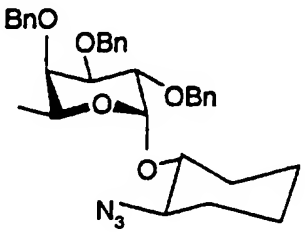
11 12

Compound 11 (122 mg, 0.12 mmol) is dissolved in methanol (2 mL), $Pd(OH)_2$ on carbon (20 mg) is added and the mixture is stirred under hydrogen (1 atm) for 12 hours. The catalyst is filtered through celite and the product is purified by silica gel chromatography ($CHCl_3$/methanol, 3:1) and biogel P2 chromatography (H_2O). Compound 12 is obtained after purification. ^1H NMR (500 MHz, D_2O) δ 1.48 (d, 3H, J = 7.0 Hz,H-6), 1.47-1.74

(m, 4H), 1.74 (s, 9H), 2.00-2.11 (m, 3H), 2.47 (m, 1H), 3.64 (s, 1H, H-4), 3.76 (m, 1H), 3.97-4.25 (m, 6H, H-2, H-3, H-5), 4.65-4.74 (m, 2H), 5.31 (d, 1H, J = 3.5 Hz, H-1). ^{13}C NMR (125 MHz, D_2O) δ 15.80, 23.73, 24.51, 28.18, 30.18, 31.60, 39.68, 49.47, 53.42, 55.89, 55.99, 62.39, 67.12, 68.42, 70.08, 72.39, 94.38, 94.65, 170.9, 171.1, 174.7, 178.1. HRMS calcd for $C_{24}H_{41}N_3O_{12}Cs$ (M+Cs+) 696.1745, found 696.1717.

Compound 11, used as starting material, may be prepared as follows:

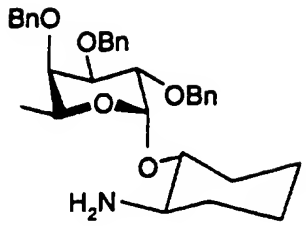
a)



8

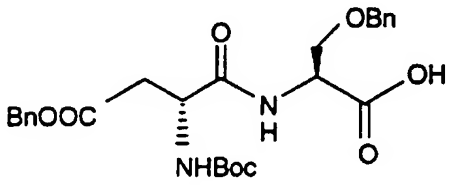
2,3,4-Tri-O-benzyl-L-fucopyranose is dissolved in anhydrous dichloromethane at 0° and DAST (1.06 g, 0.0066 mol) is dropped in. After the mixture is stirred at 0° for 30 min., the reaction is quenched by the addition of water. The aqueous solution is extracted with dichloromethane and the organic fractions are combined, dried over $MgSO_4$ and filtered. The solvent is evaporated and the fluoride is used for the glycosylation without further purification. 4Å Molecular sieves, tin (II) chloride (1.67 g, 0.0088 mol) and silver perchloride (1.82 g, 0.0088 mol) are added to a solution of the fluoride in anhydrous dichloromethane (20 mL) at 0°. The mixture is stirred for 5 min. and (R,R) azidocyclohexanol (0.93 g, 0.0066 mol) is added. The reaction is warmed to room temperature and stirred for 6 hours. After filtration through celite, the filtrate is concentrated and applied to silica gel chromatography (hexane/ethylacetate, 8:1). Compound 8 is obtained as a clear oil.

b)



9

Compound 8 (1.3 g, 2.33 mmol) is dissolved in tetrahydrofuran (10 mL, containing 1 % H_2O) and triphenylphosphine (638 mg, 2.56 mmol) is added. The mixture is stirred at room temperature for 5 h. After evaporation of the solvent, the residue is purified by chromatography on a silica gel column with $CHCl_3$/methanol (50:1 → 30:1). Compound 9 is obtained as a syrup.



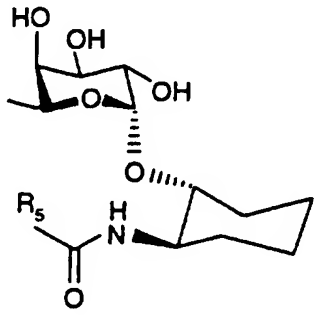
10

c) O-Benzyl-N-Boc-L-aspartic acid is dissolved in anhydrous dichloromethane and EDAC (640.3 mg, 3.34 mmol) and N-hydroxysuccinimide (384.4 mg, 3.34 mmol) are added. The mixture is stirred at 4° for 12 h and the solvent is evaporated. O-benzyl- N-Boc-aspartic N-hydroxy succinimide ester is obtained by silica gel chromatography (ethylacetate/hexane, 1:1.5 → 1:1).

O-Benzyl-N-Boc-aspartic N-succinimide ester (603 mg, 1.44 mmol) and O-benzyl-serine (281 mg, 1.44 mmol) are dissolved in DMF (2 mL) and Et_3N (1 mL) is added. The mixture is stirred at room temperature for 1 hour. After evaporation of the solvent and silica gel chromatography ($CHCl_3$) the product 10 is obtained.

d) Acid 10 (141 mg, 0.29 mmol), EDAC (81 mg, 0.42 mmol) and HOBT (57 mg, 0.42 mmol) are dissolved in dichloromethane (2 mL) at room temperature and stirred for 5 min. before compound 9 (148 mg, 0.56 mmol) is added. The mixture is stirred at room temperature for 3 hours and the solvent is evaporated. The residue is applied to a silica gel column (hexane/ethylacetate, 1.5: → 1:1) and compound 11 is obtained as a syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20 (d, 3H, J = 6.5 Hz, H-6), 1.08-1.60 (m, 4H), 1.44 (s, 9H), 1.73 (m, 1H), 1.94 (m, 3H), 2.71 (m, 1H), 3.05 (m, 1H), 3.65 (dd, 1H, J = 3.5, 9.5 Hz), 3.76 (m, 1H, H-4), 3.89 (dd, 1 H, J = 3.0, 9.5 Hz), 3.98 (dd, 1H, J = 2.5, 10.0 Hz, H-3), 4.04 (dd, 1H, J = 3.5, 10.0 Hz, H-2), 4.10 (m, 1H, H-5), 4.44 (m, 1H), 4.61 (m, 1H), 4.68 (m, 1H), 4.95 (d, 1H, J = 3.5 Hz, H-1), 4.45-5.17 (m, 10H), 5.68 (m, 1H), 7.33 (m, 25H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.72, 23.34, 23.82, 28.20, 36.29, 36.59, 50.42, 50.65, 52.45, 52.58, 52.82, 66.69, 66.72, 66.78, 69.26, 73.09, 73.18, 74.80, 75.98, 75.98, 77.73, 79.35, 94.52, 128.0 (m), 135.2, 135.3, 137.5, 183.6, 139.0, 155.3, 169.6, 170.2, 170.6, 171.9. HRMS cacld for $C_{59}H_{71}N_3O_{12}Cs$ (M+Cs+) 1146.4092, found 1146.4035.

By following a procedure in analogy with that of Example 7 above, but using the appropriate starting materials, the following compounds of formula



wherein R_5 is as indicated in Table 2, may be prepared.

Table 2

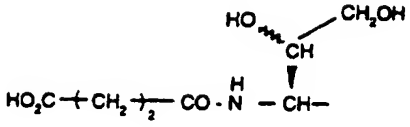
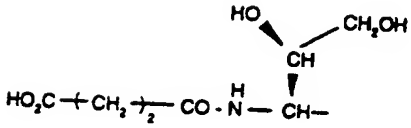
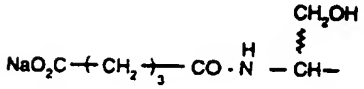
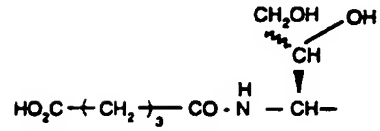
Ex.	R_5	HRMS Calc.	HRMS Found
8		[M⁺Cs⁺] 611.1217	611.1241
9		[M⁺Cs⁺] 611.1217	611.1241
10		485.2111	485.2127
11		[M⁺Cs⁺] 625.1373	625.1355

Table 2 (continued)

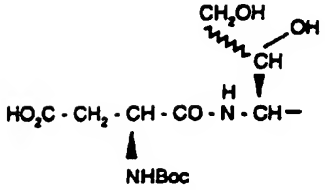
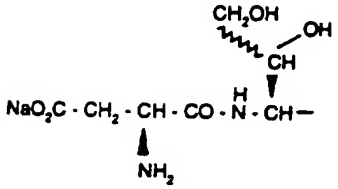
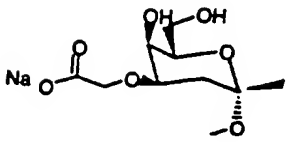
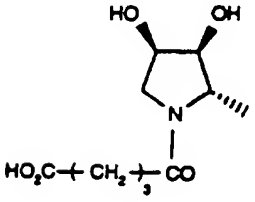
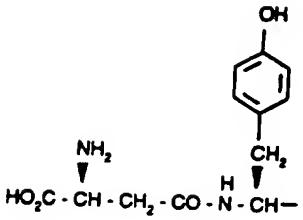
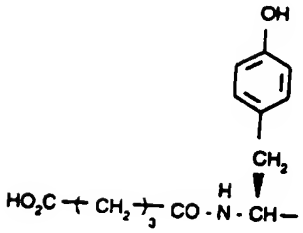
Ex.	R_5	HRMS Calc.	HRMS Found
12		[M⁺Cs⁺] 726.1850	726.1832
13		[M⁺Na⁺] 516.2169	516.2169
14			
15		[M⁺Cs⁺] 637.1373	637.1353

Table 2 (continued)

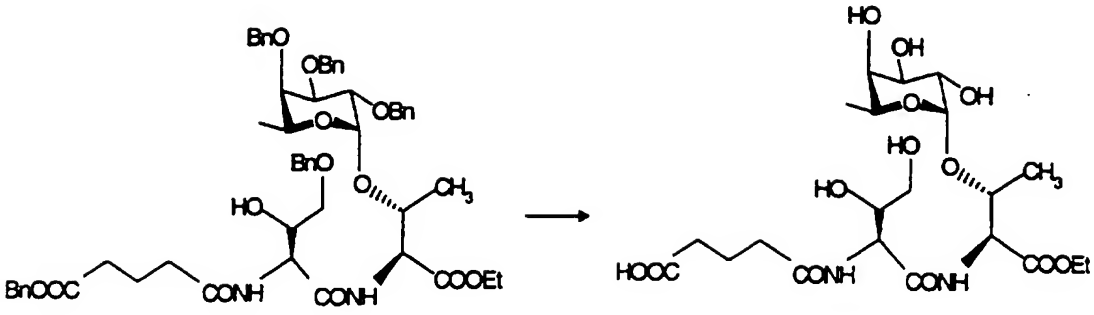
Ex.	R_5	HRMS Calc.	HRMS Found
16		[M⁺Cs⁺] 672.1533	672.1546
17			

Compound of Example 11: ^{1}H NMR (500 MHz, D_2O) δ 1.10 (d, 3H, J = 6.5 Hz, H-6), 1.20 (m, 4H), 1.75 (m, 6H), 2.16 (m, 1H), 2.56 (t, 2H, J = 7.5 Hz), 2.33 (m, 2H), 3.41 (m, 1H), 3.43 (m, 2H), 3.63 (m, 2H, H-2 and H-3), 3.69 (m, 1H, H-4), 3.78 (m, 1H, H-5), 4.09 (ddd, 1H, J = 2.5, 6.0, 8.5 Hz), 4.46 (d, 1H, J = 2.5 Hz), 5.02 (d, 1H, J = 3.0 Hz, H-1). ^{13}C NMR (125 MHz, D_2O) δ 15.85, 21.96, 23.71, 24.60, 28.84, 31.41, 35.38, 35.45, 53.67, 55.19, 62.73, 67.13, 68.26, 69.88, 71.65, 72.22, 75.19, 93.31, 172.3, 176.6.

Compound of Example 12: ^{1}H NMR (500 MHz, D_2O) δ 1.12 (d, 3H, J = 6.5 Hz, H-6), 1.08-1.26 (m, 4H), 1.39 (s, 9H), 1.65-1.80 (m, 3H), 2.14 (m, 1H), 2.74 (m, 2H), 3.39-3.88 (m, 9H), 4.37 (m, 2H), 4.98 (d, 1H, J = 3.5 Hz, H-1). ^{13}C NMR (125 MHz, D_2O) δ 15.82, 23.70, 24.58, 28.03, 29.30, 31.78, 37.32, 52.05, 53.40, 55.55, 62.93, 67.14, 68.32, 69.95, 71.99, 72.25, 76.23, 82.21, 94.06, 157.6, 170.8, 173.4, 175.8.

Compound of Example 13: ^{1}H NMR (500 MHz, D_2O) δ 1.15 (d, 3H, J = 6.5 Hz, H-6), 1.10-1.40 (m, 4H), 1.66-1.85 (m, 3H), 2.16 (m, 1H), 2.59 (dd, 1H, J = 8.5, 17.5 Hz), 2.72 (dd, 1H, J = 5.0, 17.5 Hz), 3.40 (m, 1H), 3.51 (dd, 1H, J = 6.0, 12.0Hz), 3.56 (dd, 1H, J = 3.5, 10.0 Hz, H-3), 3.60 (m, 1H), 3.64 (dd, 1H, J = 4.0, 10.0 Hz, H-2), 3.69 (m, 1H, H-4), 3.73 (m, 1H), 3.85 (m, 1H), 3.91 (m, 1H, H-5), 4.15 (m, 1H), 4.40 (d, 1H, J = 7.0 Hz), 5.00 (d, 1H, J = 4.0 Hz, H-1). ^{13}C NMR (125 MHz, D_2O) δ 15.77, 23.69, 24.62, 29.06, 31.80, 38.41, 51.47, 53.40, 55.59, 62.76, 67.09, 68.35, 69.91, 71.79, 72.24, 75.68, 93.63, 170.6, 176.8, 176.8.

Example 18:

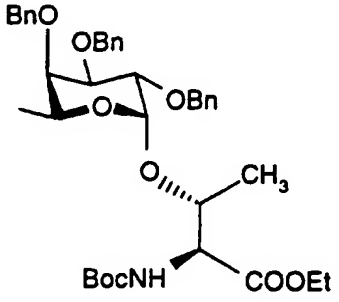


15 16

The benzyl groups of 15 are cleaved by hydrogenation according to procedure of Example 5. Compound 16 (amorphous): ^{1}H NMR (400 MHz, D_2O) δ 4.98 (d, J = 3.2 Hz, 1 H, H-1), 4.61 (d, J = 2.2 Hz, 1 H), 4.56 (d, J = 7.2 Hz, 1 H), 4.46-4.44 (dd, J = 2.2 and 6.4 Hz, 1 H), 4.28-4.13 (m, 3H), 4.02-3.98 (m, 1 H), 3.86-3.59 (m, 5 H), 2.42-2.22 (m, 4 H), 1.90-1.79 (m, 2 H), 1.30-1.18 (m, 9H); electrospray negative ion mass (declustering potential = -80 V) m/z 523 [(M-H); calcd for $C_{21}H_{36}N_2O_{13}$: 524].

Compound 15, used as starting material, may be prepared as follows:

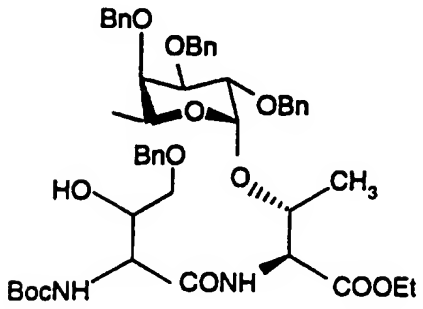
a)



13

L-Fucose is first converted to tribenzylfucosyl phosphite according to Wong et al. J. Org. Chem. 1994, 59, 864. The resulting compound (1.0 equivalent) is coupled to Boc-L-Thr-OEt (1.1 equivalents) using trifluoromethanesulfonic acid (0.1 equivalent) as catalyst in methylene chloride at 0° to give compound 13 after standard workup and purification.

b)



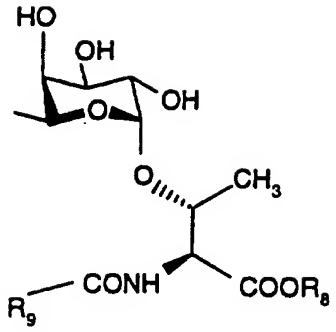
14

First Boc deprotection of 13 in 30 % TFA in 0.1 M CH_2Cl_2 at 25°, 30 min; quench water, wash $NaHCO_3$, dry sodium sulfate; purification by flash chromatography gives the corresponding free amine. This amine (1.0 equivalent) is coupled with 1.1 equivalents of (2S,3R)-N-Boc-2-amino-4-benzyloxy-3-hydroxy-butyric-acid using 1.5 equiv of EDCl, 1.5 equiv of HOBt, 0.1 M CH_2Cl_2, 0°, 30 h, to provide 14 after standard workup and flash column chromatography purification conditions.

c) Boc deprotection of 14 in 30 % TFA, 0.1 M CH_2Cl_2 at 25°, 30 min is followed by quench with water, wash with $NaHCO_3$ and drying over sodium sulfate. Purification by flash chromatography is followed by coupling with 1.1 equivalents monobenzyl glutarate, 1.5 equiv of EDCl, 1.5 equiv of HOBt, CH_2Cl_2, 25°, 20 h, to provide compound 15. Compound 15: ^1H NMR (400 MHz, $CDCl_3$) δ 8.00 (d, J = 9.1 Hz, 1 H), 7.21-7.50 (m, 25H), 6.75 (d, J = 7.6 Hz, 1 H), 5.12-4.57 (m, 11 H), 4.35-3.99 (m, 7H), 3.80-3.60 (m, 5 H), 2.45 (t, J = 7.5 Hz, 2H), 2.43 (t, J = 7.0 Hz, 2H), 1.98 (t, J = 7.5 Hz, 2H), 1.20-1.26 (m, 6 H), 1.06 (d, J = 6.4 Hz, 3 H); HRMS for $C_{56}H_{66}N_2O_{13}$+ Cs+ (M+Cs+), calcd 1107.3614, found 1107.3667.

By following a procedure in analogy with that of Example 18 above but using the appropriate starting materials, the compounds of formula

30



wherein R_8 and R_9 are as defined in Table 3, may be prepared.

Table 3

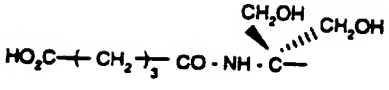
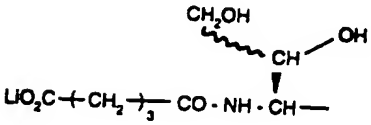
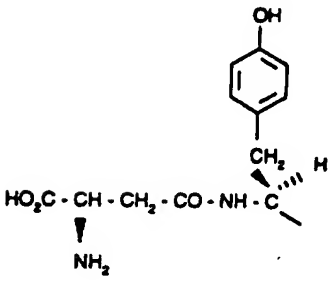
Ex.	R_8	R_9	MS calc.	MS found
19	H		ES (M-H⁻) 496	495
20	Li^+		ES (M-H⁺) 495	495
21	H		ES (M-H⁻) 543	542

Table 3 (continued)

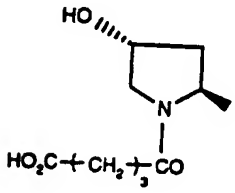
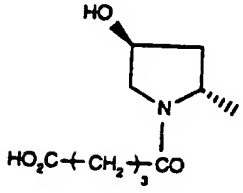
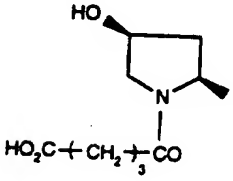
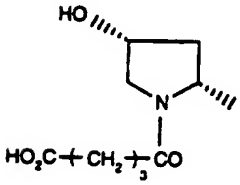
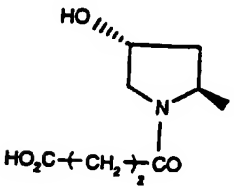
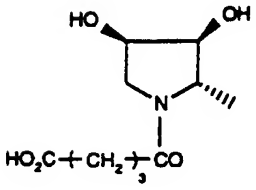
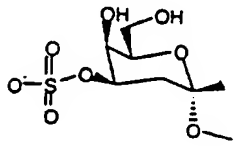
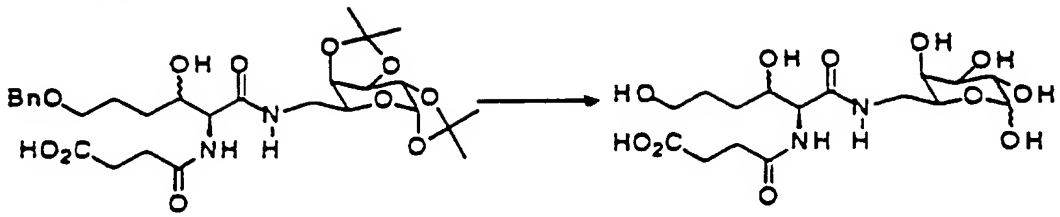
Ex.	R_8	R_9	MS calc.	MS found
22	C_2H_5		[M⁺Na⁺] 543.2166	543.2175
23	C_2H_5		[M-H⁺+2Cs⁺] 543.2166	543.2148
24	C_2H_5		[M-H⁺+2Cs⁺] 785.0299	785.0328
25	C_2H_5		[M-H]⁻ 519	519
26	C_2H_5			

Table 3 (continued)

Ex.	R_8	R_9	MS calc.	MS found
27	C_2H_5		[M+Cs⁺] 669.1272	669.1287
28	C_2H_5		[M-H+2CS] 842.9708	842.9672

Example 29



19 20

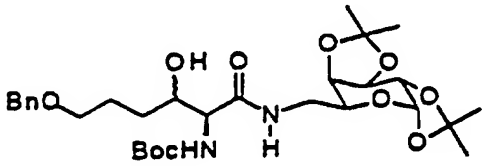
A solution of glycopeptide 19 (23.7 mg, 40 µmol) is stirred in 90 % TFA in water (1 mL) at room temperature. After 3 hrs., the reaction solution is evaporated down under reduced pressure and azeotroped twice with toluene (2 x 5 mL). An nmr on the crude product is performed to ensure that the isopropylidene moieties are removed and the product is then taken on to the next step without purification.

The crude glycopeptide is then hydrogenated according to the procedure of Example 5 to give a white solid 20: R_f (4:1:1 nBuOH:H_2O:HOAc) 0.29: ^1H nmr (400 MHz; D_2O) 5.23 (d, J 3.7, $H1_\alpha$), 4.55 (dd, J 7.9 and 4.7, $H1_\beta$), 4.37-4.32 (m, $H2'_{(\alpha+\beta)}$), 4.15-4.05 (m, $H5_\alpha$ + $H3'_\beta$), 3.95-3.92 (m, $H4_\alpha$ + $H3'_\alpha$), 3.86 (d, J 3.4, $H4_\beta$), 3.83 (dd, J 10.3 and 3.2 $H3_\alpha$), 3.78 (dd, J 10.3 and 3.7, $H2_\alpha$), 3.74-3.68 (m, $H5_\beta$) 3.64-3.61 (m,

$H3_{\beta}$ + $C6'H_{2(\alpha + \beta)}$), 3.55-3.44 (m, $H2_{\beta}$ + $C6H_aH_{b(\alpha + \beta)}$), 3.42-3.31 (m, $C6H_aH_{b(\alpha + \beta)}$), 2.62-2.48 (m, $C2"H_{2(\alpha + \beta)}$) + $C3"H_{2(\alpha + \beta)}$) , 1.76-1.47 (m, $C4'H_{2(\alpha + \beta)}$) + $C5'H_{2(\alpha + \beta)}$); ^{13}C nmr (100 MHz; D_2O) 182.93, 179.11, 175.20, 174.66, 98.86, 94.80, 75.19, 75.08, 75.02, 74.21, 72.91, 71.78, 71.48, 71.28, 71.18, 70.71, 70.52, 63.84, 63.80, 61.00, 60.90, 60.48, 42.15, 42.03, 34.71, 34.19, 34.12, 31.84, 31.44, 31.14: High Resolution Mass Spectrum (Doped with NaI): Found M + Na, 447.1570. $C_{16}H_{28}N_2O_{11}$ requires M + Na, 447.1591.

Compound 19, used as starting material, may be prepared as follows:

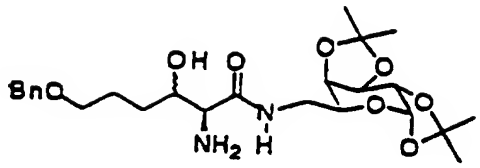
a)



17

EDCI (95.4 mg, 500 µmol) is added to a stirred solution of 6-amino-6-deoxy-1,2,3,4-diisopropylidene-α-L-galactopyranoside (130 mg, 500 µmol), (2S,3RS)-2-N-Boc-amino- 6-benzyloxy-3-hydroxy-hexanoic acid (177 mg, 500 µmol), HOBT (68 mg, 500 µmol) and 4-methyl morpholine (108 µL, 1000 µmol) in dry DMF (5 mL) under argon at -20°. The resulting mixture is stirred at -20° for 1 hr and then allowed to warm slowly to room temperature. After 14 hours, the reaction solution is quenched 5 % w/v citric acid solution (20 mL) and extracted with ethyl acetate (6 x 25 mL). The combined organic extracts are washed with saturated sodium hydrogen carbonate solution (50 mL) and saturated sodium chloride solution (50 mL), dried ($MgSO_4$) and evaporated down under reduced pressure. The residual oil is purified by flash chromatography (silica gel, using gradient elution 40% → 50% → 66% ethyl acetate in hexane) to give 17 as a pale yellow foam: R_f (75 % ethyl acetate in hexane) 0.66.

b)



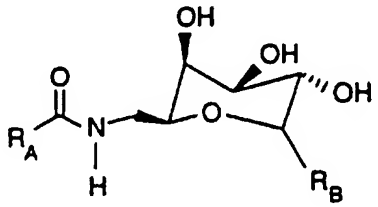
18

A solution of the glycopeptide (17) (59.5 mg, 100 µmol) in 15 % v/v trifluoroacetic acid in dry 4-(dicyanomethylene)-2-methyl-6-(4-dimethylaminostyryl)4-H-pyran (1 mL) is stirred under argon at room temperature. After 2 hr., the solution is evaporated down under reduced pressure and the residual oil dissolved up in n-butanol:water:methanol (5:3:2) (10 mL). To the solution is added Dowex (Cl^-, prewashed with methanol, 100 mg) and the mixture stirred for 30 mins., then filtered and the solid washed with methanol (3 x 5 mL) and the combined filtrate and washings evaporated under reduced pressure. The residual oil is purified by flash chromatography (silica gel, using gradient elution 19:0.9:0.1 → 9:0.9:0.1 DCM:MeOH:$NH_{3\ (aq)}$) to give 18 as a light brown oil: R_f (9:0.9:0.1 DCM:MeOH:$NH_{3\ (aq)}$) 0.42.

c) Succinic anhydride (6.0 mg, 60 µmol) is added to a stirred solution of amino glycopeptide 18 (27.7 mg, 56 µmol) in methanol (1 mL) at room temperature. After 1 hr, the solution is evaporated down under reduced pressure. The residual solid is purified by flash chromatography (silica gel, using gradient elution 5 → 10% acetic acid in ethyl acetate) to give compound 19 as a pale yellow gum. R_f (10 % acetic acid in ethyl acetate) 0.49: IR (Film) cm-1 3303, 2980, 2935, 1644, 1558, 1436, 1382, 1255, 1211, 1167, 1109, 1070, 1006, 901: ^1H nmr (400 MHz; CD_3OD) 7.32-7.23 (5H, m, aromatic), 5.44 (1H, d, J 5.0, 2 x H1), 4.59 (1H, dd, J 7.9 and 2.1, 2 x H3), 4.48 (2H, s, 2 x CH_2Ph), 4.38-4.34 (1H, m, 2 x H'2), 4.31 (1H, dd, J 5.0 and 2.4, 2 x H2) 4.21 (1H, d, J 7.9, 2 x H4), 4.00-3.89 (1.5H, m, 2 x H5 + H3'), 3.84-3.76 (0.5H, m, H3'), 3.52-3.46 (3H, m, 2 x C6H_a H_b + 2 x C6'H_2), 3.27-3.20 (1H, m, 2 x C6 H_a H_b), 2.56-2.48 (4H, m, 2 x C2"H_2 + 2 x C3"H_2), 1.80-1.54 (4H,

m, 2 x C4'H_2 + 2 x C5'H_2), 1.45 (3H, s, acetonide Me), 1.40 (3H, s, acetonide Me), 1.32 (3H, s, acetonide Me), 1.29 (3H, s, acetonide Me): ^{13}C nmr (100 MHz; CD_3OD) 137.21, 126.78, 126.24, 126.03, 107.86, 107.34, 95.19, 71.24, 70.06, 69.56, 69.28, 69.24, 68.58, 64.68, 56.66, 38.02, 28.96, 28.35, 24.54, 23.83, 23.75, 22.62, 21.99: High Resolution Mass Spectrum (Doped with CsI): Found M + Cs, 727.1875. $C_{29}H_{42}N_2O_{11}$ requires M + Cs, 727.1843.

By following a procedure in analogy with that of Example 29 but using the appropriate starting materials, the compounds of formula



wherein R_A and R_B are as defined in Table 4, may be prepared.

Table 4

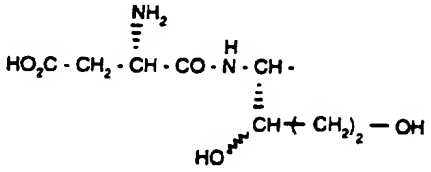
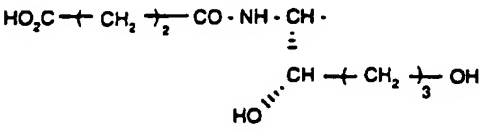
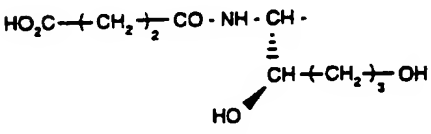
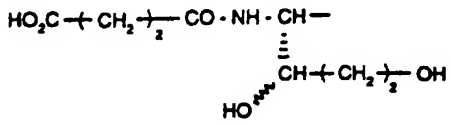
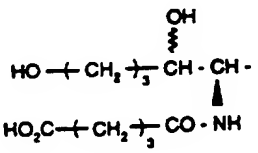
Ex.	R_A	R_B
30		
31		
32		
33		
34		

Table 4 (continued)

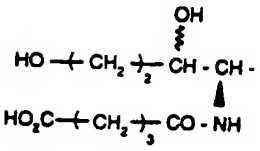
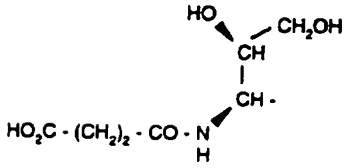
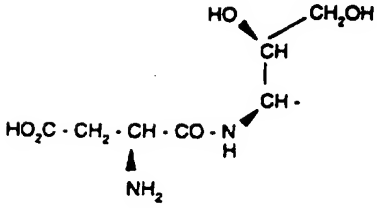
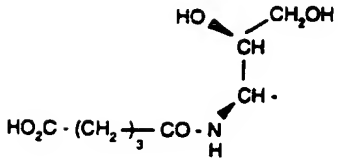
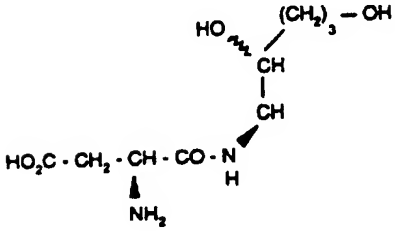
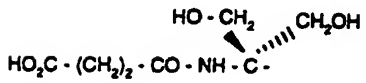
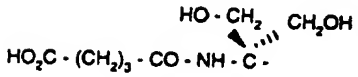
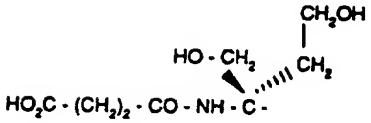
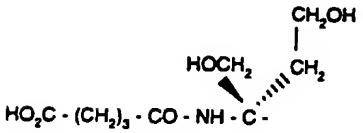
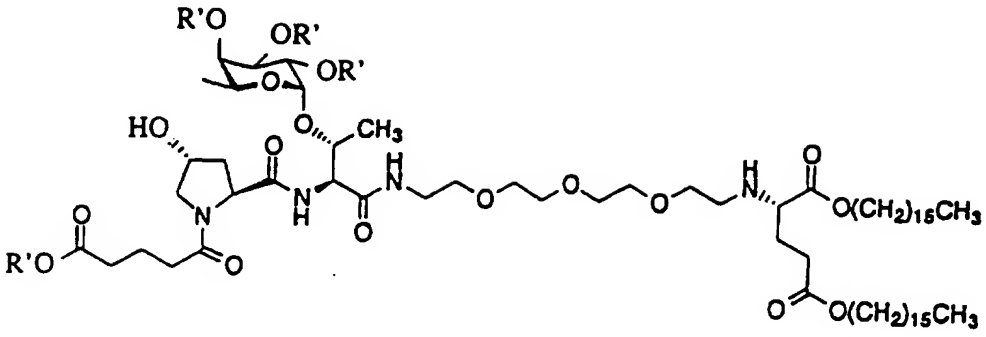
Ex.	R_A	R_B
35		
36		
37		
38		
39		

Table 4 (continued)

Ex.	R_A	R_B
40		
41		
42		
43		

Example 44



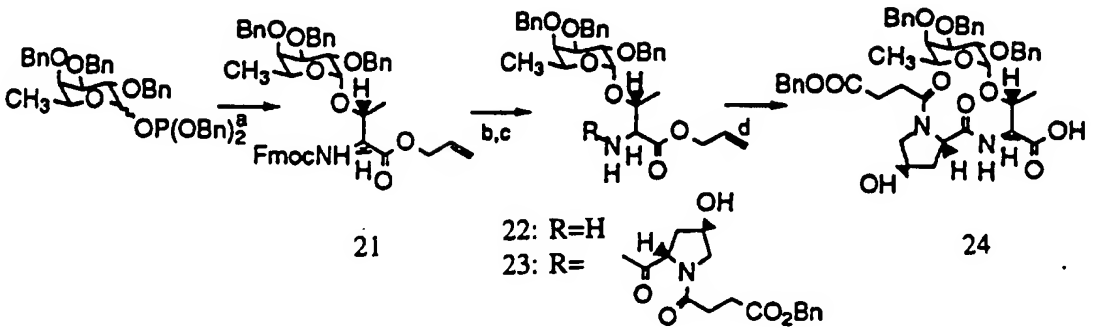
27: R' = Bn

28: R' = H

To the solution of compound 27 (91.4 mg, 59.2 μmol) in MeOH (5 mL) is added $Pd(OH)_2$ on carbon (20 mg). The mixture is stirred for 2 days under hydrogen balloon. The catalyst is filtered off and the filtrate is evaporated in vacuo. The residue is applied to silica gel column chromatography ($CHCl_3$:MeOH = 1:1) and then sephadex LH20 gel filtration chromatography (eluted with MeOH) to obtain the desired amphiphile 28. ^1H NMR (500 MHz, CD_3OD) d 0.80 (6H, t, J=6.5), 1.16 (6H, d, J=5.5), 1.23-1.41 (52H, m), 1.60-1.71 (4H, m), 2.04-2.17 (2H, m), 2.31-2.60 (6H, m), 2.66-2.77 (2H, m), 2.97-3.09 (2H, m), 3.31-3.40 (3H, m), 3.50-3.71 (14H, m), 3.79-3.87 (1H, m), 3.88-3.98 (1H, m), 4.04-4.26 (4H, m), 4.44-4.56 (3H, m), 4.95 (1H, brs). HRMS calcd for $C_{62}H_{114}N_4O_{17}Cs$ (M+Cs) 1319.7233, found 1319.7264.

Compound 27, used as starting material, may be prepared as follows:

a)



Compound 21

A suspension of dibenzyl 2,3,4-O-tribenzyl-α-L-fucopyranosyl phosphite (138 mg, 0.204 mmol), N-Fmoc threonine allyl ester (77.5 mg, 1 eq) and molecular sieve 4Å (470 mg) is stirred for 18 hr at room temperature. To the mixture is added a solution of TMSOTf (13.6 mg, 0.3 eq) in CH_2Cl_2 (1 mL) at -15°. After stirring for 2 hr at the same temperature, saturated $NaHCO_3$aq is added to quench and the mixture is diluted with CH_2Cl_2. The organic lay is separated, dried over $MgSO_4$ and evaporated in vacuo. The residue is applied to silica gel column chromatography (Hex:EtOAc = 4:1) to obtain the compound 21.

Compound 22

To a solution of compound 21 (104 mg, 0.13 mmol) in CH_2Cl_2 (5 mL) is added Et_2NH (0.72 mL, 53 eq) and the mixture is stirred for 18 hr at room temperature. The solvent and reagent are removed in vacuo and the residue is purified by silica gel column chromatography (CH_2Cl_2~CH_2Cl_2:MeOH=100:3) to obtain the compound 22.

Compound 23

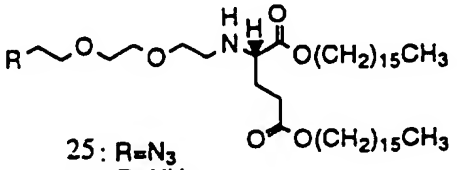
To a solution of the compound 22 (242 mg, 0.42 mmol) and N-benzyl glutarylamide-L-proline (135 mg, 1 eq) in CH_2Cl_2 (5 mL) is successively added HOBT (86 mg, 1.5 eq) and EDC (105 mg, 1.3 eq) at 0°. The reaction mixture is

stirred for 20 min at 0° and the temperature is allowed to rise to room temperature within 18 hr. The reaction mixture is evaporated in vacuo and the residue is dissolved with EtOAc. The EtOAc solution is washed with 1 N HCl, saturated $NaHCO_3$ and brine successively. The organic layer is dried over $MgSO_4$ and evaporated in vacuo. The residue is applied to silica gel column chromatography (toluene→toluene:acetone=2:1) to obtain the compound 23.

Compound 24

To a solution of the compound 23 (169 mg, 0.192 mmol) and morpholine (167 mg, 10 eq) in THF (10 mL) and DMF (1 mL) is added $Pd(PPh_3)_4$ (23 mg, 0.1 eq) under argon atmosphere at room temperature. After stirring for 24 hr, the reaction mixture is evaporated in vacuo. The residue is dissolved with EtOAc and washed with 1N HCl. The organic layer is separated, dried over $MgSO_4$ and evaporated in vacuo. The residue is purified on silica gel column chromatography (CH_2Cl_2→CH_2Cl_2:MeOH 10:1) to obtain the compound 24.

b) *Synthesis of lipid part*



25: R=N_3
26: R=NH_2

Compound 25

To a solution of oxalyl chloride (1.54 mL, 2N CH_2Cl_2 solution) is added a solution of DMSO (277 mg, 1.5 eq) in CH_2Cl_2 (3 mL) and a solution of 2-[2-(2-azidoethoxy)-ethoxy]ethanol in CH_2Cl_2 (5 mL) each 2 min interval at -78°. After the mixture is stirred for 30 min at the same temperature, Et_3N (1.64 mL) is added to the mixture at -78° and the temperature is allowed to rise to room temperature within 1 hr. The reaction mixture is poured into ice water and extracted with CH_2Cl_2. The organic

layer is washed with 1N HCl and saturated $NaHCO_3$ successively, dried over $MgSO_4$ and evaporated in vacuo. The crude aldehyde is used without further purification. To a solution of the crude aldehyde and 1',3'-dicetyl-L-glutamate p-toluenesulfonic acid salt (500 mg, 1 eq) in THF (3 mL) and MeOH (9 mL) is added $NaCNBH_3$ (41 mg) at 0°. The mixture is stirred at 0° for 1 hr and the temperature is allowed to rise to room temperature within 18 hr. The solvent is evaporated in vacuo and the residue is dissolved with CH_2Cl_2. After the solution is washed with 1 N HCl, the organic layer is dried over $MgSO_4$ and evaporated in vacuo. The residue is applied to silica gel column chromatography (Hex-EtOAc=4:1) to obtain compound 25.

Compound 26

To the solution of compound 25 (320 mg, 0.427 mmol) in MeOH (13 mL), THF (3 mL) and 1N HCl (1.3 mL) is added Pd on carbon (99 mg). The mixture is stirred for 2 days under hydrogen balloon. The catalyst is filtered off and the filtrate is evaporated in vacuo. To the residue is added saturated $NaHCO_3$ and the mixture is extracted with EtOAc. The organic layer is dried over $MgSO_4$ and evaporated in vacuo to obtain compound 26.

Coupling between sLe^x mimetic and Lipid

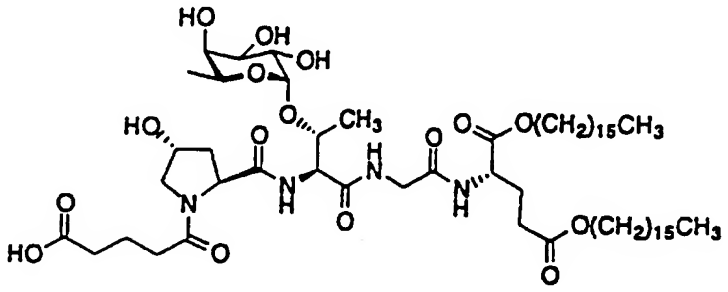
Compound 27

To a solution of compound 24 (94.6 mg, 0.113 mol) and compound 26 (81.7 mg, 1 eq) in CH_2Cl_2 (5 mL) is added HOBT (23 mg, 1.5 eq) and EDC (29 mg, 1.3 eq) successively at 0°. The reaction mixture is stirred at 0° and the temperature is allowed to rise to room temperature within 20 hr. The reaction mixture is evaporated in vacuo and the residue is dissolved with EtOAc. After the solution is washed with saturated $NaHCO_3$, the organic layer is dried over $MgSO_4$ and evaporated in vacuo. The residue is purified on silica gel column chromatography (toluene:acetone=1:1) to obtain compound 27.

^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (6H, t, J=7.0), 1.12 (3H, d, J=6.0), 1.17 (3H, d, J=6.0), 1.21-1.41 (52H, m), 1.57-1.65 (4H, m), 1.88-1.97 (2H, m), 2.12-2.20 (1H, m), 2.22-2.29 (1H, m), 2.38 (2H, t, J=7.0), 2.45-2.83 (5H, m), 3.18-3.27 (2H, m), 3.42-3.59 (10H, m), 3.63 (1H, brs), 3.66 (1H, dd, J=11.0, 4.0), 3.82 (1H, dd, J=10.3, 3.0), 3.84 (1H, q, J=7.0), 4.01-4.13 (4H, m), 4.48-4.58 (4H, m), 4.61 (1H, d, J=11.5), 4.64 (1H, d, J=11.5), 4.69 (1H, d, J=11.5), 4.74 (1H, d, J=11.5), 4.75 (1H, d, J=11.5), 4.92 (1H, d, J=11.5), 4.96 (1H, d, J=3.5), 5.06 (2H, s), 7.10 (1H, brt, J=3.0), 7.25-7.43 (20H, m), 7.87 (1H, t, J=9.0). m/z $C_{90}H_{138}N_4O_{17}$ 1548 (M+H)

Example 45

By following a procedure in analogy with that of Example 44 but using the appropriate starting materials, the following compound may be obtained



^{1}H NMR (500 MHz, CDCl3) d 4.80 (d, J=2.6 Hz, 1H), 4.46 (t, J=8.5 Hz, 1H), 4.42-4.40 (m, 4H), 4.05-3.95 (m, 6H), 3.76-3.43 (m, 6H), 2.23 (t, J=7.7 Hz, 2H), 2.30-2.20 (m, 3H), 2.12-2.01 (m, 2H), 1.98-1.91 (m, 4H), 1.85-1.77 (m, 1H), 1.57-1.50 (m, 4H), 1.25-1.13 (br., >50H), 1.11 (d, J=6.3 Hz, 3H), 1.09 (d, J=6.6 Hz, 3H), 0.78 (t, J=6.7 Hz, 6H); MS m/e calc'd for $C_{59}H_{106}N_4O_{16}Cs$ ($M+Cs^+$): 1259.6658, found 1259.6620

The compounds of formula I and their pharmaceutically acceptable salts exhibit pharmaceutical activity and are, therefore, useful as pharmaceuticals. In particular, they inhibit adhesion between cells containing a selectin such as E-selectin on their surfaces and effector cells such as neutrophils or HL-60 cells that have SLe^x on their cell surfaces, or a synthetic poly-SLe^x-product or poly-SLe^a-product. More particularly, the compounds

of formula I inhibit sLe^x binding to E-selectin as indicated in the following test methods:

a) *Preparation of a soluble form of E-selectin.* The extracellular domain of E-selectin, fused to the constant region of the κ light chain (mCκ) is cloned into the baculovirus shuttle vector pVL941 (Invitrogen) and expressed in SF-9 cells. The soluble fusion protein is purified by affinity chromatography using the rat anti-mouse Cκ monoclonal 187.1 antibody coupled to Sepharose.

Cellular Binding Assays. The compounds of formula I are assayed for their ability to block the adhesion of HL-60 cells to E-selectin immobilized onto 96 well plates. Rat anti-mouse Cκ antibody is added to 96 well plates (20 µg/ml in carbonate/bicarbonate buffer, pH 9.5) and incubated overnight at 4°C. The plates are blocked with 3 % BSA in assay buffer (20 mM HEPES, pH 7.4 containing 150 mM NaCl and 1 mM $CaCl_2$) for 8 hours at room temperature and washed 3x with assay buffer. E-selectin mouse Cκ fusion protein (10 µg/ml in assay buffer) is added and incubated for 2 hours at 37°C or overnight at 4°C. The plates are washed 3x with assay buffer. Then the compound to be assayed is added and pre-incubated for 15-30 min at 37°C. 1 x 10^5 labelled HL-60 cells in assay buffer are transferred to each well and allowed to adhere to the E-selectin for 30-45 min at 37°C. Then the plates are gently washed 3-4x with assay buffer to remove unbound cells. Adherent cells are quantified by measurement of fluorescence (Cytofluor 2350 system).

Fluorescent labelling of HL-60 cells with BCECF-AM: HL-60 cells are cultured in Iscove medium supplemented with 20 % FCS, glutamine and non essential amino acids. One day before the experiment is performed, the cells are subcultured (1x10^6c/ml). The cells (1 x 10^6c/ml) are labelled by incubating with 5 µg/ml BCECF-AM (diluted from stock in DMSO) for 20 minutes at 37°C in PBS.

Materials: ELISA plate: Nunc Immuno Plate MaxiSorp (439454)
HL-60 cells: obtained from ATTC catalogue
Affinity purified E-selectin: each batch of E-selectin is tested functionally to determine the appropriate concentration for use in the assay.

Fluorescent dye: bis-carboxyethyl-carboxyfluorescein acetoxy methyl ester (BCECF-AM) available from Molecular Probes.

In this assay, compounds of formula I inhibit adhesion of HL-60 cells to E-selectin, at a concentration of from 0.3 to 10 mM. Compounds of Examples 12, 13 and 18 inhibit adhesion of HL-60 cells to E-selectin at a rate of 100 % and 80 % (for the 2 later) in a concentration of 10 mM.

b) *sLe^a-polymer/E-selectin cell free binding assay.* Rat anti-mouse Cκ antibody (10 µg/ml in carbonate/bicarbonate buffer, pH 9.5), prepared from rat hybridoma cell line 187.1 (ATCC) is coated onto microtiter plates overnight at 4°C. The plates are washed two times with assay buffer (20 mM HEPES, pH 7.4 containing 150 mM NaCl and 1 mM $CaCl_2$), blocked for 8 hours at 4°C with 3 % BSA in assay buffer and washed 3 times. To each well E-selectin mouse Cκ fusion protein (3 µg/ml in assay buffer) is added followed by an incubation overnight at 4°C. A complex is formed between biotinylated sLe^a-polymer (polyacrylamide-type glycoconjugate, Syntesome GmbH, Munich, Germany) containing 20 % mol sLe^a (= 0.81 µmol sLe^a/mg polymer) and streptavidin-peroxidase (Boehringer Mannheim, Germany) by incubating 20 µl of sLe^a-polymer (1 mg/ml) with 80 µl streptavidin-peroxidase, 20 µl fetal calf serum and 80 µl assay buffer without $CaCl_2$ for two hours at 37°C (the pre-formed sLe^a-polymer/streptavidin-peroxidase complex is stable over several months at 4°C). The complex is then diluted 3:10'000 in assay buffer and added to the E-selectin coated wells together with the compound to be assayed. The complex is allowed to bind for two hours at 37°C before the plates are washed twice with cold assay buffer. ABTS peroxidase substrate (Biorad) solution is added to the wells and the reaction is stopped after 10 min. Bound sLe^a-polymer complex is determined by measuring the optical density at 405 nm in a microplate reader. In this assay, compounds of formula I inhibit the sLe^a-polymer/E-selectin binding interaction when used at a concentration of from 0.07 to 10 nM.

Compounds of Examples 12, 13 and 18 inhibit the SLe^a-polymer/E-selectin binding interaction at an IC_{50} (50 % inhibition) of 1 mM, 10 mM and 0.5 mM, respectively.

c) *In vivo assays*. A compound of formula I may be used as a one-for-one replacement for SLe^x in vivo treatments, e.g. as described in the rat/cobra venom model by Mulligan et al., Nature, 364: 149-151 (1993) or in the feline model of myocardial ischaemia/reperfusion injury by Murohara et al., Cardiovascular Research 30, 965-974 (1995). Compounds of formula I show a beneficial effect in these models.

The compounds of formula I are, therefore, useful in the treatment and/or prevention of disorders or diseases which are mediated by the binding of selectins in cellular adhesion, particularly E-selectin, e.g. acute or chronic inflammatory or autoimmune diseases such as rheumatoid arthritis, asthma, allergy conditions, psoriasis, contact dermatitis, adult respiratory distress syndrome, inflammatory bowel disease and ophthalmic inflammatory diseases, infection diseases such as septic shock, traumatic shock, thrombosis and inappropriate platelet aggregation conditions, cardiovascular diseases such as heart attacks, reperfusion injury, multiple sclerosis and neoplastic diseases including metastasis conditions, strokes and acute or chronic rejection of organ or tissue transplants.

For the above uses the required dosage will of course vary depending on the mode of administration, the particular condition to be treated and the effect desired. In general, however, satisfactory results are achieved at dosage rates of from about 0.5 to 80 mg/kg animal body weight. Suitable daily dosage rates for larger mammals, for example humans, are of the order of from about 100 mg to 1.5 g/day, conveniently administered once, in divided dosages 2 to 4 x / day, or in sustained release form. Unit dosage forms suitably comprise from about 25 mg to 0.750 g of a compound of formula I, together with a pharmaceutical acceptable diluent or carrier therefor.

Compounds of formula I may be administered in free form or in pharmaceutically acceptable salt form. Such salts may be prepared in conventional manner and exhibit the same order of activity as the free compounds. The present invention also provides a pharmaceutical composition comprising a compound of the invention, in free base form or in pharmaceutically acceptable salt form in association with a pharmaceutically acceptable diluent or carrier. Such compositions may be formulated in conventional manner. Compounds of the invention may be administered by any conventional route,

for example parenterally e.g in form of injectable solutions or suspensions, or in a nasal or a suppository form.

In accordance with the foregoing the present invention further provides:

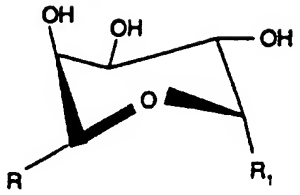
a) a compound of the invention or a pharmaceutically acceptable salt for use as a pharmaceutical;

b) a method for preventing or treating disorders as indicated above in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound of formula I or a pharmaceutically acceptable salt;

c) a compound of the invention or a pharmaceutically acceptable salt for use in the preparation of a pharmaceutical composition for use in the method as in b) above.

CLAIMS

1. A compound of formula I



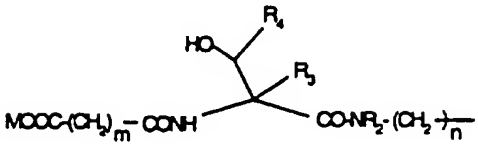
I

wherein

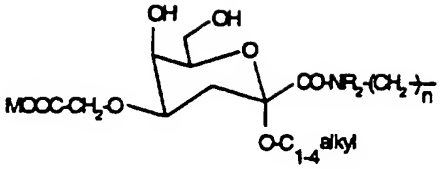
i) R is CH_3, and

either

R_1 is a radical of formulae (a_1) or (a_2)



(a_1)



(a_2)

wherein

m is 2 or 3;

n is 2 or 3;

M is a cation;

R_2 is H or a saturated or unsaturated hydrocarbon residue with up to 20 carbon atoms, optionally bearing in ω position a formyl or a C_{1-4} alcohol acetal or C_{2-4} diol acetal group;

R_3 is H, $-CH_2OH$ or $-CH_2CH_2OH$; and

R_4 is H, C_{1-4}alkyl, $-CH_2OH$, $-CH_2CH_2OH$ or $-CH_2CH_2CH_2OH$ with the provisos that

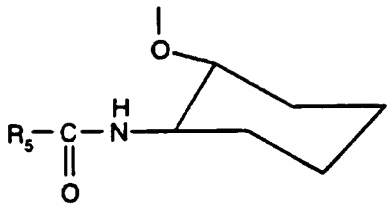
1) one of R_3 and R_4 is H, and

2) when R_4 is H, R_3 is $-CH_2OH$ or $-CH_2CH_2OH$, and

3) when R_3 is H, R_4 is CH_3, $-CH_2OH$, $-CH_2CH_2OH$ or $-CH_2CH_2CH_2OH$;

or

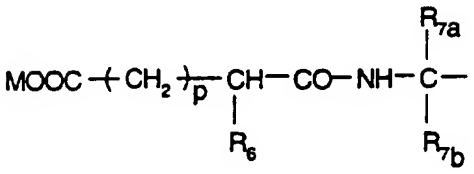
R_1 is a radical of formula (b)



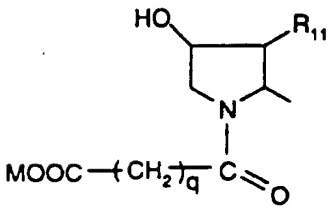
(b)

wherein

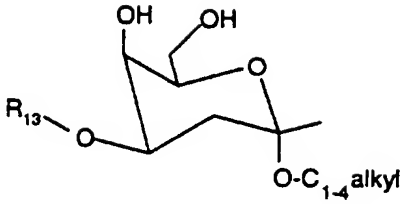
R_5 is



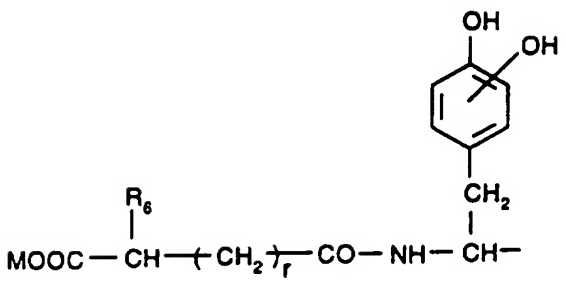
(b_1)



(b_2)



(b_3)



or (b4)

wherein

p is 1 or 2;

q is 2 or 3;

r is 1 or 2;

R_6 is H, NH_2 or $-NHR_x$ wherein R_x is an amino protecting group;

R_{7a} is $-CH_2OH$, $-CH_2CH_2OH$ or $-CH(OH)-CH_2OH$ and R_{7b} is H or each of R_{7a} and R_{7b} is CH_2OH;

R_{11} is H or -OH;

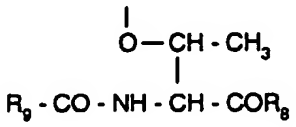
R_{13} is $-(CH_2)_j-COOM$ or $-SO_3M$ wherein j is 1, 2 or 3; and

M is as defined above;

the second hydroxy substituent of the phenyl group in (b_4) being in either meta position;

or

R_1 is a radical of formula (c)

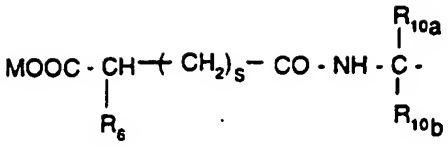


(c)

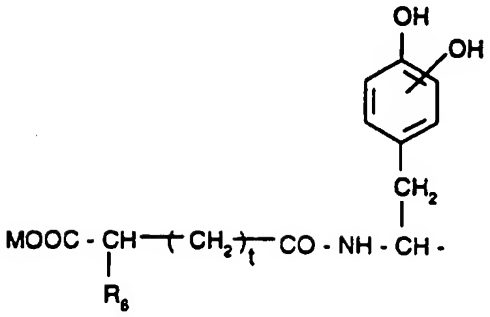
wherein

R_8 is OM_1, OR_{14}, R_s-R_p or NHR_y wherein M_1 is a cation, R_{14} is a saturated or unsaturated hydrocarbon residue, R_s is a spacer group, R_p is a phosphatidyl residue and R_y is a lipophilic residue; and

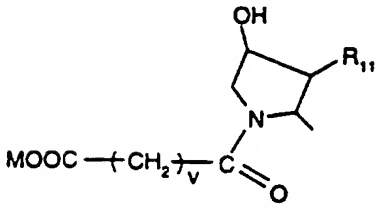
R_9 is



(c1)

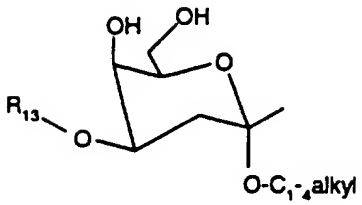


(c2)



(c3)

or



(c4)

wherein

s is 1 or 2;

t is 1 or 2;

v is 2 or 3;

M, R_6, R_{11} and R_{13} are as defined above; and

R_{10a} is $-CH_2OH$, $-CH_2CH_2OH$ or $-CH(OH)-CH_2OH$ and R_{10b} is H

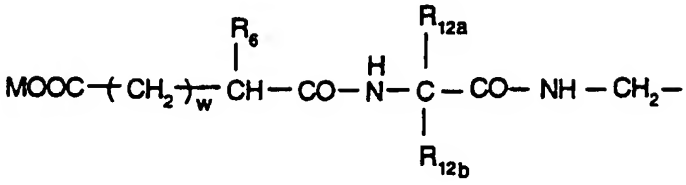
or each of R_{10a} and R_{10b} is CH_2OH;

the second hydroxy substituent of the phenyl group in (c_2) being in either meta position;

or wherein

ii) R_1 is OH, and

R is a radical of formula (d)



(d)

wherein

w is 1 or 2;

R_{12a} is $-CH(OH)-(CH_2)_x-OH$ and R_{12b} is H or each of R_{12a} and R_{12b} independently is $-CH_2OH$ or $-CH_2CH_2OH$;

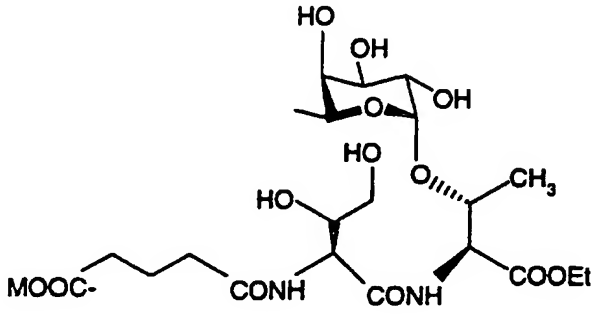
x is 2 or 3; and

R_6 and M are as defined above.

2. A compound of formula I according to claim 1 wherein R is CH_3 and R_1 is a radical of formula (c), R_9 being a radical (c_1) or (c_3).

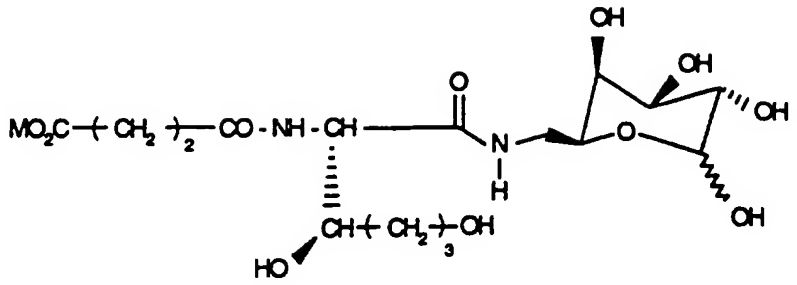
3. A compound of formula I according to claim 1 wherein R_1 is OH and R is a radical of formula (d).

4. A compound of formula

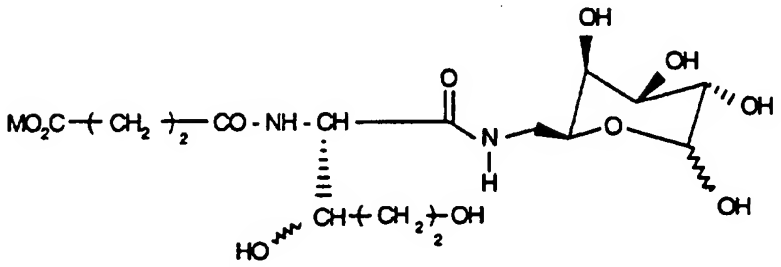


wherein M is a cation.

5. A compound of formula

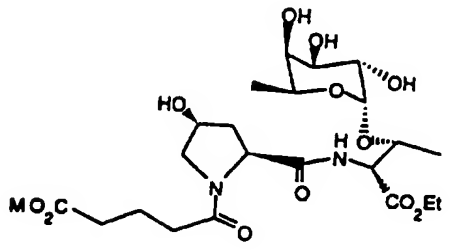


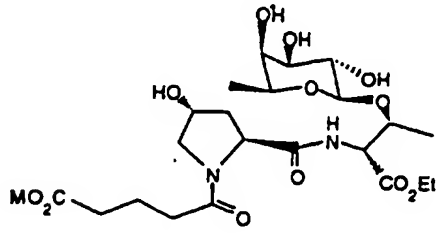
or



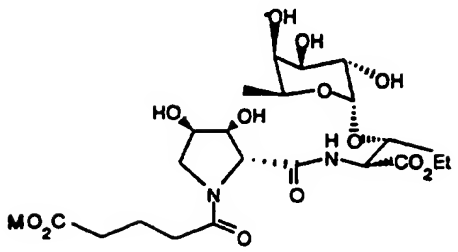
wherein M is a cation.

6. A compound of formula





or



wherein M is a cation.

7. A process for the production of a compound of formula I according to claim 1 comprising removing at least one protecting group which is present in a compound of formula I in protected form, and where required, recovering the compounds of formula I thus obtained in free form or in salt form.

8. A compound according to any one of claims 1 to 6 for use as a pharmaceutical.

9. A pharmaceutical composition comprising a compound according to any one of claims 1 to 6, together with a pharmaceutically acceptable diluent or carrier therefor.

10. A method for preventing or treating disorders or diseases which are mediated by the binding of selectins in cellular adhesion in a subject in need of such treatment, which method comprises administering to said subject an effective amount of a compound according to any one of claims 1 to 6.

INTERNATIONAL SEARCH REPORT

Int ıtional application No.
PCT/EP 96/01244

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 9/00, C07H 7/02, A61K 31/70, A61K 38/14
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Angew.Chem., Int.Ed.Engl., Volume 35(1), 1996, C.-H. Wong et al.,"Synthesis of Fucopeptides as Sialyl Lewis X Mimetics", pages 88-90 --	1-2,4,7-9
X,P	J.Am.Chem.Soc., Volume 117, 1995, C.-H. Wong et al., "Design and Synthesis of Sialyl Lewis X Mimetics", pages 5395-5396 --	1-2,7-9
X,P	J. Org.Chem., Volume 60, 1995, H. Huang and C.-H. Wong, "Synthesis of Biologically Active Sialyl Lewis X Mimetics", pages 3100-3106 --	1-2,7-9

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 1996	30.07.96
Name and mailing address of the International Searching Authority European Patent Office, P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Carolina Gómez Lagerlöf

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int. ational application No.
PCT/EP 96/01244

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J. Biol.Chem., Volume 269, 1994, B.N. Narasinga Rao et al., "Sialyl Lewis X Mimics Derived from a Pharmacophore Search Are Selectin Inhibitors with Anti-inflammatory Activity", pages 19663-19666	1-10

INTERNATIONAL SEARCH REPORT

Int ational application No.
PCT/EP 96/01244

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.